# ENHANCEMENT OF CELL BOUNDARIES IN TRANSMISSION ELECTRON MICROSCOPY IMAGES

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## ABSTRACT

Transmission electron microscopy (TEM) is an important modality for the analysis of cellular structures in neurobiology. The computational analysis of neurons entail their segmentation and reconstruction from TEM images. This problem is complicated by the heavily textured nature of cellular TEM images and typically low signal-to-noise ratios. In this paper, we propose a new partial differential equation for enhancing the contrast and continuity of cell membranes in TEM images.

## 1. INTRODUCTION

An important goal of neurobiological research is to decipher the patterns of neuronal connections that govern vertebrate behavior. Medical imaging modalities such as magnetic resonance imaging (MRI) provide 3D measurements of the brain with resolutions on the order of 1 mm. This resolution provides macroscopic information about brain organization, but does not allow analysis of individual neurons. Confocal light microscopy provides 3D measurements with a resolution on the order of 1 micron, which is still insufficient to reconstruct synaptic connections of individual neurons. Understanding brain function at the cellular level requires a detailed analysis of connectivity of individual neurons in regions that consist of densely packed cells and processes. Electron microscopy, which can provide resolutions on the order of 1 nanometer, remains the primary tool for resolving neurons, their sub-cellular 3D structures, and their connections. However, compared to the vast amount of research in medical imaging modalities such as MRI and CT, the number of scientific papers on electron microscopy applications in the image processing community has been very limited.

Detailed, data-driven descriptions of cells are especially important in neurobiology [1, 2]. Several methods have been proposed in the literature for the segmentation and reconstruction of neurons from transmission electron microscopy (TEM) images. Vazquez *et al.* introduced a semi-automatic, differential geometric method for segmenting neurons in EM images [3]. In their method, a user initializes points on the boundary of the neuron, then a minimal length geodesic criteria is used to complete the boundary. Deformable curve model based segmentation methods have also been proposed [4, 5]. All of the methods discussed above use cell membranes in some way to direct the segmentation; hence, they would benefit from a filtering approach that can reduce noise while enhancing membrane structures.

## 2. RELATED WORK

In TEM, a thin specimen is cut and stained, then is suspended in an electron beam. The staining agent, which blocks the electron beam, is selectively picked up by different structures such as membranes. The source of contrast in TEM images is the darker appearance of stained structures, which include cell and organelle membranes, and other structures such a synaptic vesicles in images of neuronal cells. Cells can be segmented by detecting their membranes; however, this process is complicated by the texture generated by other stained structures. In this paper, we propose a partial differential equation (PDE) for enhancing the contrast and continuity of cell membranes in TEM images.

Perona and Malik proposed a non-linear diffusion PDE which restricts diffusion around image edges [6]. This PDE is particularly suitable for denoising images composed of piecewise homogeneous intensity regions. It fails in textured images and more generally, in images with structures that do not conform to the piecewise homogeneity assumption. As will be shown in Section 4, the Perona and Malik PDE is not suitable for filtering TEM images.

In TEM images, cell membranes appear as noisy, one-dimensional structures (curves), and in 3D they appear as sheets. A PDE that can be used to enhance curvilinear coherence in images was proposed by Weickert [7]. This PDE performs directional diffusion aligned with anisotropic structures in images. The image anisotropy is computed using intensity gradient distributions of local neighborhoods

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(structure tensor). A PDE based on the structure tensor was used to remove speckle noise from ultrasound images [8]. Carmona and Zhong proposed using the eigenvalues of the Hessian to limit the diffusion around features [9]; however, their method is for denoising only and is not a coherence-enhancing PDE. In this paper, we propose a modification of Weickert's coherence enhancing diffusion by replacing the information from the structure tensor by information from the Hessian (Section 3). This new PDE is then used to enhance cell membranes is TEM images and compared against the previously discussed PDEs (Section 4).

## 3. METHODS

The principles of coherence enhancing diffusion was introduced in [7]. Given an image $f(\mathbf{x}) : \Omega \rightarrow \mathbb{R}$, a filtered version $u(\mathbf{x}, t)$ is computed as the solution to the following PDE:

$$\frac{\partial u}{\partial t} = \nabla \cdot (\mathbf{D} \nabla u), \tag{1}$$

with the initial condition $u(\mathbf{x}, 0) = f(\mathbf{x})$ and homogeneous Neumann boundary conditions on $d\Omega$. The diffusion tensor $\mathbf{D}$ specifies the anisotropic directionality of the diffusion. The scale parameter $t$ determines the extent of filtering.

Let $u_\sigma$ denote the convolution of $u$ with $K_\sigma$, a Gaussian kernel with standard deviation $\sigma$. Let $K_\rho$ be another Gaussian kernel with standard deviation $\rho$. Then a structure tensor can be defined as $K_\rho * (\nabla u_\sigma \otimes \nabla u_\sigma)$ [7]. Typically, $\sigma$ is 1 pixel or less, whereas $\rho$ is chosen to define a neighborhood size. Then, this tensor summarizes the first order structure of neighborhoods: two small eigenvalues in flat intensity areas, one large eigenvalue around linear structures (ridges and edges) and two large eigenvalues at junctions, corners and peaks. Weickert defined the diffusion tensor $\mathbf{D}$ to have the same eigenvectors as the structure tensor, but with modified eigenvalues that are chosen to concentrate diffusion along the directions of linearity, i.e. tangential to an edge. Let $\mu_1$ and $\mu_2$ be the eigenvalues of $\mathbf{D}$ with $\mu_1 \geq \mu_2$. Then the eigenvalues of $\mathbf{D}$ are chosen as

$$\lambda_1 := \alpha \text{ and } \lambda_2 := \alpha + (1-\alpha), \exp\left(-C/(\mu_1 - \mu_2)^2\right) \tag{2}$$

where $C$ is a threshold parameter and $\alpha$ is a regularization parameter that introduces a limited amount of isotropic diffusion to the system. Typical values for $C$ and $\alpha$ are 1 and 0.001, respectively. While, this choice of $\mathbf{D}$ leads to a coherence enhancing PDE, we found that constructing the diffusion tensor based on the Hessian instead of the structure tensor gave better results when applied to cellular TEM images (see Section 4). Define a Gaussian filtered version of the Hessian:

$$H_\rho := \begin{pmatrix} \frac{\partial^2 u_\rho}{\partial^2 x} & \frac{\partial^2 u_\rho}{\partial x \partial y} \\ \frac{\partial^2 u_\rho}{\partial x \partial y} & \frac{\partial^2 u_\rho}{\partial^2 y} \end{pmatrix}, \tag{3}$$

where $\rho$ defines a neighborhood size as in the case of the structure tensor. Let $\mu_1$ and $\mu_2$ be the eigenvalues of $H_\rho$. Then we define the diffusion tensor $\mathbf{D}$ to have the same eigenvectors as $H_\rho$, and with eigenvalues defined as in equation 2. The numerical implementation of the diffusion PDE is beyond the scope of this paper, and is given in detail in [7].

## 4. EXPERIMENTS

Figure 1(a) shows a portion of a TEM image of a rabbit retina. Membranes of neurons and organelles inside the neuron cells, such as micro-filaments and vesicles, appear darker due to the staining process. The enhancement of cell membranes requires: (i) closing of gaps in the membranes due to non-uniform staining, and (ii) removal of noise and enhancement of contrast. Filtering with a Gaussian kernel removes noise; however, it also blurs membranes, Figure 1(b). As expected, the Perona and Malik PDE [6] moves the input image toward a piecewise constant image, Figure 1(c). The continuity of membranes is not enhanced; in fact, it is even disrupted in certain places such as the long, horizontally oriented cell on the right side of the image. The geometry of the membranes and the texture inside the cells make this PDE unsuitable for cellular TEM images.

The results of Weickert's coherence enhancing diffusion PDE is shown in Figure 3(a). We experimented with the parameters to obtain the best qualitative results ($\alpha = 0.001, \sigma = 1.0, C = 1.0, \rho = 3.0, t = 40$). The continuity of membranes is enhanced, but their contrast from surrounding textures is not improved. The eigenvector of the structure tensor with the smaller eigenvalue is aligned with linear structures (edges, ridges) by construction. Figure 2(a) shows the angle of this eigenvector; it does not closely match the membrane structures.

The second-order derivative response of ridge-like features is stronger than their first-order derivative response. Carmona and Zhong proposed a diffusion PDE with a scalar diffusion coefficient based on the smaller eigenvalue of the Hessian [9]. However, their method differs from ours in two important respects: (i) the Hessian is not convolved with a smoothing kernel as in equation 3 and (ii) their approach is for limiting the diffusion around linear features only; it is not a explicitly directional, coherence-enhancing PDE. In this paper, we propose to use the directionality (eigenvectors) as well as the eigenvalues of the Hessian to substitute the structure tensor in Weickert's coherence enhancement framework. Figure 2(b) and (c) show the angle of the Hessian eigenvector (for a small portion of the image) with the smaller eigenvalue (in magnitude) computed with $\rho = 0$ (no smoothing) and $\rho = 3.0$, respectively. These results illustrate that smoothing of the Hessian is essential before the computation of eigenvectors. The parameters used

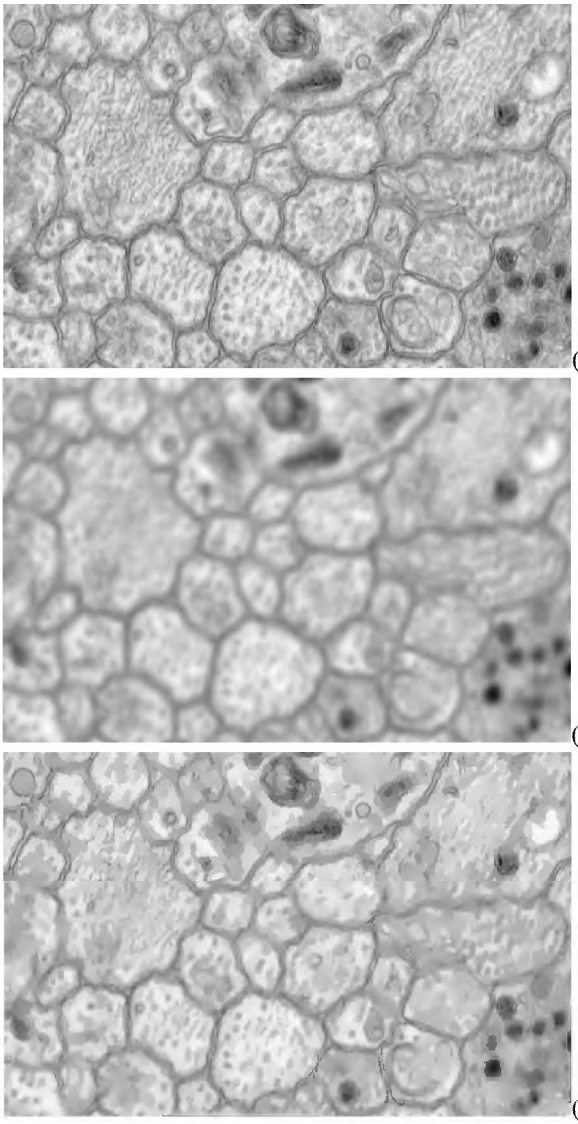


**Fig. 1**. (a) A portion of a TEM image of a rabbit retina, and results of processing with (b) Gaussian filtering, (c) Perona-Malik PDE.

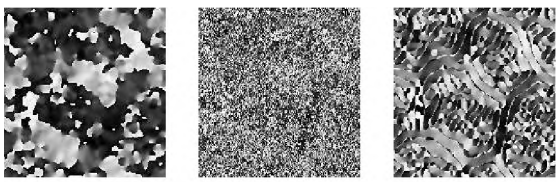


**Fig. 2**. Angle of eigenvector (for a small portion of the TEM image) from (a) the structure tensor, (b) Hessian with no smoothing, and (c) Hessian with smoothing $\rho = 3$ pixels.

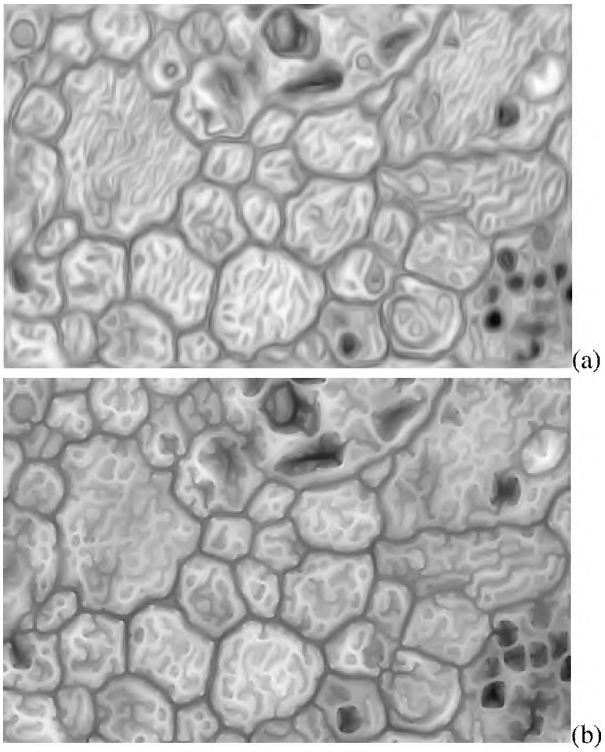


**Fig. 3**. (a) Weickert PDE and (b) proposed Hessian PDE.

are the same as in the structure tensor computation, i.e the same $\rho$ is used resulting in the same amount of averaging in both cases. Compared to the angle from the structure tensor, the angle from the Hessian follows the directions of the membranes much more closely. This better representation of membrane angles results in a better enhancement result shown in Figure 3(b). This result (image size $528 \times 339$) took approximately 3 minutes to compute on a high-end single-processor PC.

In Figure 3(b), the contrast of the membranes and their continuity are enhanced. To verify the contrast enhancement claim, we performed the following membrane detection experiment. Membranes are among the darkest objects in the image. Figure 4(a-c) show the intensity histograms of the original image, the image processed with the Weickert PDE and the proposed PDE. Figure 4(d-f) show segmentation by simple intensity thresholding. The original histogram hints at a subtle two-class intensity distribution, but this is smoothed into a single class distribution by the Weickert PDE. No threshold value can produce a satisfactory result for detecting membranes. On the other hand, the histogram of the image processed with the proposed method has a clear two-class nature. Due to the better diffusion alignment along membranes, their intensities are not mixed with the brighter surroundings. Consequently, a threshold can be chosen to detect membranes with a relatively small

amount of false-positives. More complicated algorithms could be applied to the processed image for better detection results.

## 5. CONCLUSIONS

In this paper, we introduced a new way of enhancing the contrast of cell membranes in TEM images. We adopt the framework of Weickert's coherence enhancing PDE. However, the diffusion directions are computed from a smoothed Hessian matrix (second-order derivative structure) rather than the structure tensor (first-order derivative structure). The second-order derivatives are better indicators of elongated, thin structures in images; therefore, better filtering results are obtained with the proposed method. In this paper, this is demonstrated in the context of enhancing membranes of cells in TEM images which is very important for neurobiological research that aims to reconstruct shapes and connectivities of neurons. Other applications with similar image features, such as road finding, can also benefit from using the proposed method as a pre-processing step.

**Acknowledgements:** This research was partially supported by NSF EIA0313268, NIH EY002576, and NIH EY015128.

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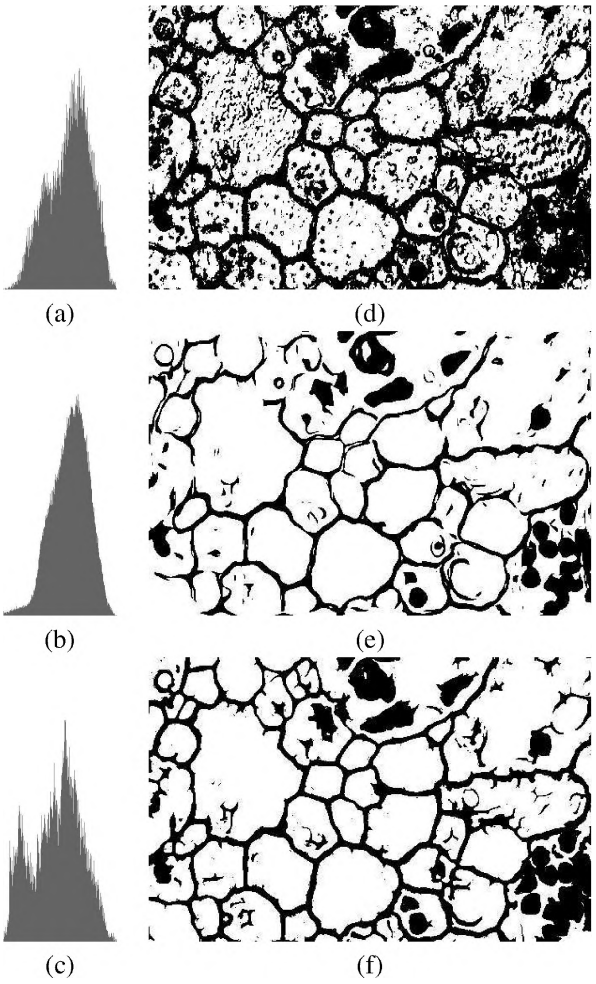


**Fig. 4**. Feature detection: Histograms of (a) original image, (b) Weickert's PDE, and (c) proposed Hessian PDE. Results of thresholding the respective images: (d-f).

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